diseases. We have characterized the lipid-induced aggregation of tau by FCS. We find that aggregation occurs when the amount of protein bound to the lipid bilayer exceeds a critical surface density. Our results suggest that the lipid bilayer facilitates protein-protein interactions both by screening charges on the protein as well as by increasing the local protein concentration, resulting in rapid aggregation. Our work highlights the versatility of FCS in studying this important class of proteins.

**98-Symp**
**Total Internal Reflection with Fluorescence Correlation Spectroscopy**
**Nancy Thompson**.
University North Carolina, Chapel Hill, Chapel Hill, NC, USA.
The combination of total internal reflection illumination with fluorescence correlation spectroscopy (TIR-FCS) allows one to examine in quantitative detail a variety of biophysical properties related to the motions and interactions of fluorescent molecules near the interface of a transparent planar surface and an adjacent solution. Several experimental and theoretical aspects of this combination will be discussed. TIR-FCS has allowed characterization of local diffusion coefficients and concentrations of fluorescently labeled antibodies in solution but very close to substrate-supported phospholipid bilayers. TIR-FCS has also been used to examine the interaction kinetics of fluorescently labeled mouse IgG specifically and reversibly associating with the mouse receptor Fc-gamma-RII, which was purified and reconstituted into substrate-supported planar membranes. This method also has the potential, through the use of a single fluorescent reporter, of providing information about the thermodynamics/kinetics of nonfluorescent molecules which participate in surface binding mechanisms; e.g., those that compete with fluorescent reporters for surface-immobilized receptors or those that interact on the surface with the receptors and reduce or enhance the interaction of the fluorescent reporters with the surface binding sites.

**99-Symp**
**Measuring Biomolecular Interactions with Single Wavelength Fluorescence Cross-Correlation Spectroscopy**
**Thorsten Wohland, Ph.D.**
Chemistry, National University of Singapore, Singapore, Singapore.
Biomolecular interactions are strongly influenced by the complex cellular environment in which molecules will interact not only with each other but often compete with or interact via other molecules. Therefore it is necessary to establish methods which can quantitatively measure interactions within the environment of cells and organisms. Fluorescence Cross-Correlation Spectroscopy (FCCS) is a widely used tool for this purpose. FCCS determines interactions by exciting two fluorescently tagged molecules with different excitation and emission spectra. It uses two distinct laser lines, which have to be brought to the same focus, for excitation. But in tissues, where aberrations can be significant, the overlap of the focal volumes can be strongly position and time dependent rendering quantification difficult. In single wavelength FCCS (SW-FCCS) a single laser line is used to excite two fluorophores with similar excitation but distinct emission spectra making the alignment of two lasers unnecessary. SW-FCCS has been shown to work with quantum dots, tandem dyes, and organic fluorophores. But in particular it can be applied to fluorescent protein pairs, e.g. GFP and mRFP/mCherry, which facilitates its application in live samples. Since its inception SW-FCCS has been applied in live cells and organisms to determine receptor dimerization, monitor phosphorylation of activated receptors, and determine biomolecular affinities. We will discuss advantages and pitfalls of SW-FCCS and will show its extension to an imaging format. Confocal SW-FCCS can measure interactions only at single spots. A more accurate picture of the interactions happening simultaneously in a cell can be obtained by performing SW-FCCS in an imaging format where hundreds of points can be measured simultaneously. We therefore extended SW-FCCS to camera based FCCS to measure interactions at multiple points in a cell.

**100-Symp**
**40 Years of FCS - A Success Story in Biology**
**Petra Schwille**.
BIOTEC, TU Dresden, Dresden, Germany.
Four decades after its invention, Fluorescence Correlation Spectroscopy has found its way into the canon of standard methods for the quantitative analysis of biological systems. Originally designed for the study of fast molecular dynamics modulating the fluorescence emission of molecules, and for a long time being exclusively applied in well-equilibrated buffer solutions, a large body of instrumental and methodological improvements have paved the way for its rigorous aplication in the study of complex living systems. After briefly reviewing some of the most important milestones in FCS development, I will discuss challenges and prospects for its potential future use in systems biology.

## Minisymposium: Allosteric Communication in Ring-shaped ATPases

**101-MiniSymp**
**Lis1 Uncouples Allosteric Communication Between Dynein's AAA+ Motor and Microtubule Binding Domains**
**Samara L. Reck-Peterson**[1], Julie Huang[1], Anthony J. Roberts[1], Andres E. Leschziner[2].
[1]Harvard Med Sch, Boston, MA, USA, [2]Harvard University, Cambridge, MA, USA.
Cytoplasmic dynein, the large microtubule-based motor protein, is highly regulated in cells, enabling it to be deployed to specific sites, collect cargos, and translocate them towards the microtubule minus-end at specific times. Defects in the regulation of dynein can cause neurological diseases; for example, mutations in the dynein co-factor Lis1 cause lissencephaly. Dynein's ''engine'' is evolved from ring-shaped AAA+ ATPases, and its microtubule-binding domain lies at the tip of a coiled-coil stalk, however how these elements might be acted upon to achieve control remains unknown. Here, using purified proteins from S. cerevisiae, we dissect how Lis1/Pac1 and its binding partner Nudel/Ndl1 control cytoplasmic dynein. At the single molecule level, we find that Lis1 slows dynein's velocity and prolongs its microtubule encounters. Nudel allows Lis1 to function at lower concentrations, suggesting it acts as a tether between Lis1 and dynein. High precision analysis shows that Lis1 causes dynein to adopt a microtubule-anchored state by uncoupling dynein's ATPase cycle from its binding to microtubules, such that ATP is consumed without the usual microtubule release and forward motion. To understand the structural basis for Lis1 regulation, we mapped its binding site on dynein using electron microscopy and 2D image analysis. Unexpectedly, we find that Lis1 binds at the interface between dynein's AAA+ ring and microtubule-binding stalk. Mutational analyses are suggestive of electrostatic interactions at this dynein-Lis1 interface, which are disrupted by lissencephaly-causing mutations. We propose that Lis1 regulates the transmission of structural changes between dynein's AAA+ ring and microtubule binding stalk, biasing dynein towards a microtubule-anchored state. In vivo this could allow Lis1 to assist in dynein's microtubule plus-end localization and cargo loading, and prevent slippage during dynein's tension-bearing roles.

**102-MiniSymp**
**Separable Roles for ATPase Domains Involved in Virulence Factor Secretion**
**Briana Burton**.
Harvard University, Cambridge, MA, USA.
Pathogenic bacteria utilize specialized protein secretion machinery in order to either affect their host cells or to alter the bacterial cell surface itself. One such emerging secretion system, originally described in Mycobacterium tuberculosis, has been termed the Type VII secretion system (T7S) in bacteria. This system is defined in part by the presence of membrane-associated FtsK/SpoIIIE-like AAA-ATPase domain containing proteins, each containing multiple active sites. While presumed to be involved in the secretion of the virulence proteins, the function of the ATPases has not been previously determined. Furthermore, the broader family of these secretion systems includes gene clusters where the FtsK/SpoIIIE-like protein is split and coded by two genes rather than one. Here we explore the role of these ATPases in protein secretion using two T7S systems, one with a single ATPase and the other with a naturally split ATPase. Using complementary biochemical and in vivo assays, we demonstrate that the ATPases are essential for protein secretion. Further, we find that the active sites contribute differentially to substrate interaction and export, thus suggesting a novel system in which a subset of the ATPase domains may function as their own adapter molecules.

**103-MiniSymp**
**Allosteric Regulation of Nucleotide Binding to the Proteasomal ATPases**
**David M. Smith**[1], Hugo Fraga[2], Christian Reis[2], Alfred Goldberg[2].
[1]WVU School of Medicine, Morgantown, WV, USA,
[2]Harvard Medical School, Boston, MA, USA.
Protein degradation by the eukaryotic 26S proteasome or the homologous archaeal PAN-20S proteasome complex is a multistep process that requires ATP hydrolysis by the proteasome-associated AAA ATPase complex. However, the mechanisms by which these hexameric ATPases bind and hydrolyze ATP to promote protein breakdown are poorly understood. Although

PAN contains six identical ATPase subunits, we found that it exhibits three types of ATP binding sites: 2 high affinity conformations (ATP-binding sites), 2 with lower affinity (ADP-binding sites), and 2 with conformations that do not bind nucleotides. Correlation of ADP off rates with rates of ATP hydrolysis suggests that ADP leaving may be the rate limiting step in hydrolysis. ATP binding to the high and lower affinity sites has distinct functional consequences on the proteasome. With two ATPγS molecules bound, PAN maximally stimulates opening of the gated channel for substrate entry into the 20S proteasome and has a high affinity for the 20S. However, the binding of 4 ATPγS reduces PAN's affinity for the 20S, which can be explained by steric hindrances in the PAN-20S interface. Because ATP binding drives the association of the C-termini of the ATPase with the 20S and only two ATPase subunits bind ATP for maximal function it's likely that only two ATPases' C-termini dock into the 20S at any time and in a predictable pattern mirroring the cyclical pattern of ATP hydrolysis. This observation suggests how the symmetrically mismatched hexameric ATPase ring associates with the heptameric 20S proteasome to regulate substrate degradation.

**104-MiniSymp**
**Eliminating ATP Binding in Specific ClpX Subunits Yields Functional ATP-Fueled Protein-Unfolding Machines**
**Benjamin M. Stinson**, Steven E. Glynn, Andrew R. Nager, Tania A. Baker, Robert T. Sauer.
MIT, Cambridge, MA, USA.
AAA+ ring hexamers of ClpX utilize the energy of ATP binding and hydrolysis to unfold protein substrates and translocate the resulting denatured polypeptide into the degradation chamber of ClpP, an associated self-compartmentalized peptidase. Nucleotide-dependent conformational changes are necessary for ClpX binding to ClpP and to protein substrates as well as for allosteric machine function. ClpX functions asymmetrically. In crystal structures, for example, four loadable subunits are competent for nucleotide binding, whereas two unloadable subunits are not. Moreover, ATP hydrolysis in one subunit can power conformational changes in the entire ring and protein degradation. Subunit-subunit interactions are essential for ClpX machine function, but the communication mechanisms are poorly defined. For instance, it is not known whether the conformations of loadable and unloadable subunits remain fixed or interchange during the hundreds of cycles of ATP hydrolysis that are required for protein unfolding, translocation, and degradation. To probe subunit communication, we constructed covalently linked mutant hexamers in which the nucleotide affinity of specific subunits was dramatically reduced by mutations in the Walker A, sensor-II, or box II motifs and developed novel fluorescence assays to probe nucleotide-binding cooperativity as well as ATP binding to specific subunits. Strikingly, ClpX pseudo-hexamers bearing two opposed subunits with severe ATP-binding defects hydrolyze ATP at near normal rates and are able to unfold and translocate protein substrates. For some variants, machine function was retained despite sensor-II-dependent abrogation of the positive cooperativity of ATP binding. Because hexamers with two ''permanent'' unloadable subunits retain basic ClpX machine functionality, subunit switching between unloadable and loadable conformations does not appear to be required for protein unfolding or translocation. Our results are most consistent with probabilistic models of ATP binding and hydrolysis rather than strictly sequential models.

**105-MiniSymp**
**The Structure of the Dynein Motor Domain**
**Andrew P. Carter**.
MRC Lab of Molecular Biology, Cambridge, United Kingdom.
Dyneins are microtubule-based motor proteins that power ciliary beating, transport intracellular cargos, and help to construct the mitotic spindle. Evolved from ring-shaped hexameric AAA-family adenosine triphosphatases (ATPases), dynein's large size and complexity have posed challenges for understanding its structure and mechanism. Here, we present a 6 angstrom crystal structure of a functional dimer of two ~300-kilodalton motor domains of yeast cytoplasmic dynein. The structure reveals an unusual asymmetric arrangement of ATPase domains in the ring-shaped motor domain, the manner in which the mechanical element interacts with the ATPase ring, and an unexpected interaction between two coiled coils that create a base for the microtubule binding domain. The arrangement of these elements provides clues as to how adenosine triphosphate-driven conformational changes might be transmitted across the motor domain.

**106-MiniSymp**
**The Fellowship of the Ring**
**Francis T.F. Tsai**, Amadeo B. Biter, Jungsoon Lee, Nuri Sung, Sukyeong Lee.
Baylor College of Medicine, Houston, TX, USA.
AAA+ ATPases are a group of conserved, ring-forming, ATP-dependent molecular machines that harness the energy of ATP binding and hydrolysis to drive diverse biological activities, ranging from protein unfolding to DNA translocation. While common mechanisms are apparent, distinct structural features exist that confer specific functions. Yeast Hsp104 and its bacterial ortholog ClpB are ATP-dependent protein disaggregases, which, together with the cognate Hsp70 system, rescue stress-damaged proteins from a previously aggregated state. The ability to do so is strictly dependent on the M-domain that forms an 85-Å long coiled-coil and is a hallmark of the ClpB/Hsp104 family. While substrate translocation through the ClpB hexamer is essential for protein disaggregation, it remains unclear how ATP is coupled to the power stroke that drives protein unfolding and translocation. At this mini-symposium, I will present our latest, unpublished data on the structure, mechanism, and function of ATP-dependent protein disaggregases.
This work was supported by grants from the National Institutes of Health (AI076239, GM067672, and RR002250) and the Robert A. Welch Foundation (Q-1530).

## Platform: Computational Methods

**107-Plat**
**Equilibrium Sampling using a Weighted Ensemble of Dynamical Trajectories**
Carsen Stringer, Matthew Zwier, Lillian Chong, **Daniel Zuckerman**.
Univ Pittsburgh, Pittsburgh, PA, USA.
The ''weighted ensemble'' (WE) method, originally designed for nonequilibrium path sampling, can also be applied to equilibrium sampling [J. Chem. Phys. 133: 014110 (2010)]. WE is a parallel method with multiple trajectories coupled periodically through configuration space in a statistically rigorous way. We demonstrate the first applications of equilibrium WE to molecular systems. Because ''ordinary'' dynamics trajectories are employed, the approach can simultaneously yield rate constants for transitions among arbitrary states.

**108-Plat**
**Simple and Efficient Calculation of Scattering Intensities of Proteins in Solution from Atomistically Detailed Structures**
**Juergen Koefinger**, Gerhard Hummer.
NIH/NIDDK, Bethesda, MD, USA.
Solution scattering experiments provide signatures of the atomistic structure of proteins, nucleic acids, and biomolecular assemblies under near physiological conditions. Coarse-grained structural properties like shape and volume can be inferred from an essentially model-free analysis using information in the small-angle regime. The wide-angle regime offers higher resolution information which can be interpreted using atomistic models. A combination of time-resolved scattering experiments, molecular simulations, and ensemble refinement methods helps reveal structural changes in proteins as they perform their biological functions. To address this challenge, we developed a mathematically simple and computationally efficient method to calculate the scattering intensity of atomistically detailed structures of proteins in solution. Compared to other methods, our method, which is based on Debye's formula, has the advantage that there is no trade-off between computational efficiency and accuracy in the promising wide-angle regime. We present results for a variety of proteins and different water models and discuss some fundamental differences in interpretation of small- and wide-angle data.

**109-Plat**
**The Simultaneous Determination of Diffusion Coefficients and PMFs Through the OFR Method**
**Bryan W. Holland**[1], Bruno Tomberli[2], Chris G. Gray[1].
[1]University of Guelph, Guelph, ON, Canada, [2]Brandon University, Brandon, MB, Canada.
Progress in nonequilibrium, bidirectional work theorems have lead to the development of an important theory, known as the forward-reverse (FR)